

CLAIMS:

1. A method for identifying active substances which affect the covalent bonding of polypeptides to the surface of Gram-positive bacteria, comprising the following steps:
 - a) providing a sample of Gram-positive bacteria which contain or produce at least one enzymatic reporter substance which is or can become covalently bonded to the surface of the Gram-positive bacteria, said at least one reporter substance having a different enzymatic activity when not covalently bonded to the surface of the Gram-positive bacteria from that exhibited when it is covalently bonded to the surface of the Gram-positive bacteria;
 - b) contacting the sample with a possible active substance; and
 - c) assaying the enzymatic activity of the reporter substance of the Gram-positive bacteria of the sample.
2. The method according to claim 1, characterized in that said assaying of the enzymatic activity of the reporter substance is done by comparison with at least one reference sample which has not been genetically altered, and/or at least one reference sample in which the reporter substance is non-covalently bonded to the surface of Gram-positive bacteria, and/or at least one reference sample in which the reporter substance is covalently bonded to the surface of the Gram-positive bacteria, and/or at least one reference sample in which the reporter substance is pres-

ent without covalent bonding to the surface of the Gram-positive bacteria.

a 3. The method according to ~~claims 1 and/or 2~~ characterized in that said covalent bonding of the polypeptides is effected to the murein of the cell wall, especially at interpeptide bridges, such as pentaglycines, of Gram-positive bacteria.

a 4. The method according to ~~at least one of claims 1 to 3~~, characterized in that said polypeptides are pathogenicity factors of Gram-positive bacteria.

a 5. The method according to ~~at least one of claims 1 to 4~~, characterized in that said reporter substance is a hybrid polypeptide.

B 6. The method according to claim 5, characterized in that said hybrid polypeptide has a succession of the following sequence segments: N-terminal signal peptide, enzyme, sequence segment having the sequence LPXTG, hydrophobic sequence segment, and charged sequence segment.

7. The method according to claim 6, characterized in that said enzyme is provided as a proenzyme.

a 8. The method according to ~~at least one of claims 6 to 7~~, characterized in that said change of enzymatic activity is due to a transition of the enzyme from an inactive to an active conformation or vice versa.

a 9. The method according to claim 6 to 8, characterized in that a linker peptide, especially one comprising less than 10 amino acids, is provided between said enzyme and said sequence segment having the sequence LPXTG.

10. The method according to ~~at least one of claims 1 to 9,~~ characterized in that said Gram-positive bacteria have a low natural cell wall turnover and/or a small number of cell wall proteases and/or a small number of secreted proteases.

11. The method according to ~~at least one of claims 1 to 10,~~ characterized in that said assaying of the enzymatic activity of said at least one reporter substance is done using fluorescence spectroscopy, especially confocal fluorescence spectroscopy

12. The method according to ~~at least one of claims 1 to 11,~~ characterized in that said Gram-positive bacteria express Lif.

13. The method according to ~~at least one of claims 1 to 12,~~ characterized in that the fraction of reporter substances released by natural cell wall changes is determined.

14. The method according to ~~at least one of claims 1 to 13,~~ characterized in that the fraction of reporter substances which are non-covalently bonded to the surface of Gram-positive bacteria is determined.

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